Genic divergence in *Spalacopus cyanus*
(Rodentia, Octodontidae)

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Abstract

Studied electrophoretic variation of proteins encoded by 23 loci in four populations of *Spalacopus cyanus*. Mean polymorphism and heterozygosity were 18.5 % and 5.8 %, respectively. Low levels of interpopulational genetic differentiation were found ($\overline{S}$ = 0.93, $\overline{D}$ = 0.032). Fixation index indicated a considerable degree of demic structuration (F_{ST} = 0.273), contrary to the inference that *Spalacopus* forms extensive interbreeding populations. Levels of interpopulational allozymic differences do not support the niche-width variation hypothesis. The amount of genic variation depends on the degree of isolation between local populations. Isolation in turn is influenced by physiographic features and historical chance events, and is not correlated to the geographic distance per se.

Introduction

Allozymic polymorphisms in subterranean rodents are interpreted as the stochastic outcome of factors including historical events and population structure (Patton 1980; Patton and Smith 1989), or as an adaptive consequence stemming from natural selection (Nevo 1990; Nevo and Shaw 1972). Historical events associated with the breeding structure (Patton 1980), and evlutionary constraints imposed by the fossorial way of life (Nevo 1979; Savic and Nevo 1990) have been invoked to explain the patterns of genetic variation in *Thomomys bottae* (Patton and Yang 1977), *T. umbrinus* (Patton and Feder 1978), and *Geomys bursarius* (Penney and Zimmerman 1976; Bohlin and Zimmerman 1982). Thus, gene flow is thought to be the causative agent responsible for patterns of genic and chromosomal variation as reflecting historical biogeography (Patton and Yang 1977). Alternatively, an adaptive strategy directed by natural selection operating in a monotonous subterranean niche has been proposed for *Spalax ehrenbergi* (Nevo and Shaw 1972), *Thomomys talpoides* (Nevo et al. 1974), and for the Bathyergidae (Nevo et al. 1987).

Subterranean *Spalacopus* is a monotypic genus of endemic herbivorous rodents (Contreras and Gutiérrez 1991) distributed throughout the western slope of the Andes of central Chile (Reise and Gallardo 1989a). Animals depict the same populational attributes (Reig 1970) and physiological adaptive syndrome (Contreras 1986) described for other underground mammals (Nevo 1979). Preliminary ecological studies in *Spalacopus* (Reig 1970) provided a basis for correlating the inferred wandering nature of the animals with karyotypic stability (Reig et al. 1972). In this study we examine the patterns of allozymic variation and degree of genetic fragmentation in four natural populations of *Spalacopus cyanus* from their coastal range. Low levels of interpopulation genetic differentiation as predicted by the high vagility attributed to *Spalacopus* are compared with electrophoretic data to test the consistency of such an ecology-based proposition.

Material and methods

Electrophoretic analysis was carried out on 76 specimens belonging to four populations of *Spalacopus cyanus*. Sample designations, geographic coordinates, and number of specimens examined were as follows (Fig. 1): *Spalacopus cyanus cyanus*, Los Vilos (31° 55′ S, 71° 31′ W), 28; Los Cristales (31° 55′ S, 71° 29′ W), 7; Huentelauquén (31° 35′ S, 71° 32′ W), 10. *Spalacopus cyanus maulinus*, Quirihue (36° 17′ S, 72° 32′ W), 31. Voucher specimens were deposited in the Collection of Mammals, Institute of Ecology and Evolution, Universidad Austral of Chile.
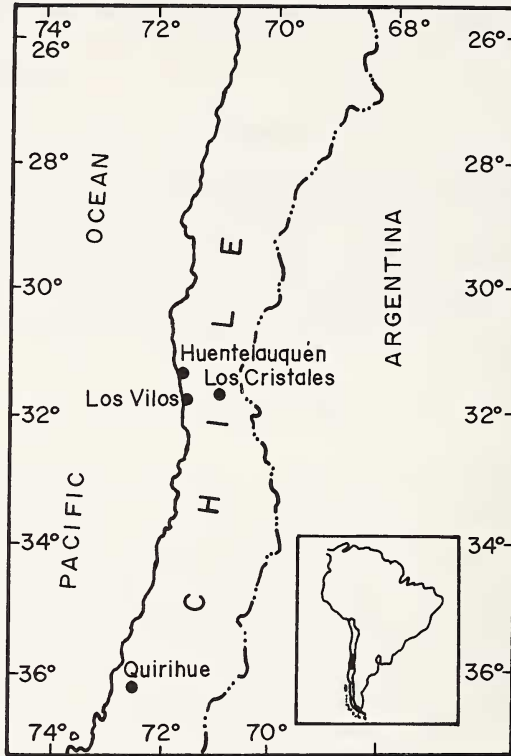

Fig. 1. Map of collecting localities of *Spalacopus cyanus*

Kidneys and liver were removed from each specimen and stored in liquid nitrogen after sacrifice. Homogenates, buffer systems, migration conditions, and mixtures for each system were prepared according to SELANDER et al. (1971). Fifteen enzymes and two non-enzymatic protein (albumin and transferrin) encoded by 23 presumptive genetic loci were examined. Details on the enzymes analysed, their tissue source, and electrophoretic procedures used are given in Table 1. Allozymes were named alphabetically according to their mobility relative to the commonest allele. A locus was considered polymorphic if the frequency of the most common allele did not exceed 0.95. D-statistics were computed to test for deficiency or excess of heterozygosity (SWOFFORD and SELANDER 1989). NEI's (1978) unbiased distance (D) and ROGERS' similarity (S) coefficients (ROGERS 1972) were calculated to compare pairs of samples. The genetic relationships among populations were represented by a dendrogram, using the weighted and the unweighted pair-group cluster algorithm (SNEATH and SOKAL 1963). An analysis of heterogeneity between pairs of populations was conducted by a contingency table using the Pearson chi-square statistic (SWOFFORD and SELANDER 1989). Spatial correlation in allele frequencies was examined by three distribution-free permutational approachs (MANTEL, KENDALL and SPEARMAN) that compare genetic and geographic distance matrices (DIETZ 1983).
Population subdivision was assessed through WRIGHT's F-statistics (WRIGHT 1965). All computations were performed in Biosys-1 (SWOFFORD and SELANDER 1989). The criterion of SLATKIN (1981) for minimum sample size (n = 10) was used to correct sampling bias of FST.

Table 1. **Enzymes, tissue sources and electrophoretic conditions resolved in** *Spalacopus cyanus*

Enzyme	Enzyme commision number	Locus abbreviation	Electrophoretic conditions and tissue source[1]
Isocitrate dehydrogenase	1.1.1.42	ICD-1	4 (K)
	1.1.1.42	ICD-2	4 (K)
Malate dehydrogenase	1.1.1.37	MDH-1	4 (K)
	1.1.1.37	MDH-2	4 (K)
Glutamate-oxaloacetate transaminase	2.6.1.1	GOT-1	5 (L)
	2.6.1.1	GOT-2	5 (L)
Glycerol-3-phosphate dehydrogenase	1.1.1.8	GPD	5 (L)
Glucose 6-phosphate dehydrogenase	1.1.1.49	Gd	6 (K)
Lactate dehydrogenase	1.1.1.27	LDH-1	3 (K)
	1.1.1.27	LDH-2	3 (K)
Xanthine dehydrogenase	2.7.1.1	XDH	3 (K)
Glucose phosphate isomerase	5.3.1.9	GPI	7 (L)
Phosphoglucomutase	2.7.5.1	PGM-2	7 (L)
	2.7.5.1	PGM-3	7 (L)
Phosphogluconate dehydrogenase	1.1.1.44	PGD	7 (L)
Alcohol dehydrogenase	1.1.1.1	ADH-1	9 (L)
	1.1.1.1	ADH-2	9 (L)
Hexokinase	2.7.1.1	HK	9 (L)
Malic enzyme	1.1.1.40	ME-1	5 (L)
	1.1.1.40	ME-2	5 (L)

[1]from SELANDER et al. (1971). Tissue source (L) = liver, (K) = kidney.

Results

Eight of the 23 loci analyzed (GOT-1, TRFER, PGI-2, PGM-2, PGM-3, ADH-1, ADH-2, HK) were polymorphic in one or more populations whereas the remainder were monomorphic across all populations (Tab. 2). Four of the variable loci (GOT-1, PGM-2, PGM-3, ADH-2) were monomorphic in two or three populations.

The average number of alleles per locus ranged from 1.1 (Quirihue) to 1.3 (Huentelauquén and Los Vilos). Polymorphism per population fluctuated from 8.7 % (Quirihue) to 26.1 % (Los Vilos), with 19.6 % for overall polymorphism across all populations. Direct-count heterozygosity per population ranged from 0.6 % (Quirihue) to 7.3 % (Los Vilos), with an unweighted mean across all samples of 4.5 %.

D-statistics indicated a deficiency of heterozygotes in loci PGM-3 and ADH-1, and total absence of heterozygotes in loci GOT-1, PGI-2, and ADH-2, suggesting that some populations are influenced by nonrandom mating and small effective population sizes (BARROWCLOUGH 1980). Fixation of otherwise polymorphic alleles (GOT-1, TRFER, PGM-2, PGM-2, ADH-1) was observed in the Quirihue sample.

High values of genetic similarity, ranging from 0.972 (Los Cristales – Los Vilos) to 0.900 (Quirihue – Huentelauquén) were found. The genetic distance ($\overline{X}$ = 0.032) ranged from 0.001 (Los Vilos – Huentelauquén) to 0.074 (Quirihue – Huentelauquén). The non-significant results (P ≥ 0.079) otained in the tests of association between geographic and genetic distance matrices indicated a nearly random distribution of genotypes (EPPERSON and ALLARD 1989).

The UPGMA and the WPGMA phenetic clustering procedures using genetic distances gave similar results, identifying two genetic subgroups (Fig. 2). The first was formed by the three northern samples, with Los Vilos and Los Cristales depicting the closest affinity. The second group was formed by the Quirihue sample, removed from the rest by the fixation of specific alleles (TRFER, ADH-1) that were polymorphic in the other samples

Table 2. Allele frequencies of eight variable loci of four populations of *Spalacopus cyanus*

| Locus | Population | | | |
	Quirihue (N = 31)	Los Vilos (N = 28)	Los Cristales (N = 7)	Huentelauquen (N =10)
GOT-1				
A	.000	.036	.000	.000
B	1.000	.964*	1.000	1.000
TRFER				
A	.000	.481	.214	.300
B	1.000	.519	.786	.700
PGI-2				
A	.000	.000	.000	.300
B	.516	.179	.143	.300
C	.484*	.821*	.857*	.400*
PGM-2				
A	1.000	.679	.786	.100
B	.000	.321	.214	.900
PGM-3				
A	.000	.054	.071	.000
B	1.000	.946*	.929	1.000
ADH-1				
A	.000	.685	.643	.800
B	1.000	.315*	.357	.200
ADH-2				
A	.000	.036	.000	.000
B	1.000	.964*	1.000*	1.000
HEXOK				
A	.065	.107	.000	.100
B	.935	.893	1.000	.900

* Significantly different from the Hardy-Weinberg equilibrium.

(Tab. 1). The test for genetic heterogeneity between populations indicated significant differences between every pair of populations except Los Vilos and Los Cristales ($P \leq 0.37$), which were considered homogeneous by the test.

The mean FST was 0.273, indicating extensive genetic subdivision, despite the low genetic distances recorded. This means that 27 % of the total variance in allelic frequencies is expressed between the populations (ZINK and WINKLER 1983). If the Quirihue sample is removed from the analysis, maximum variance in allele frequency drops to FST = 0.140. Since an inverse correlation exists between Nm and FST, this indicates that an average of one individual every second generation is exchanged among populations (HARTL and CLARK 1989). The inbreeding coefficient (FIS = 0.307) permitted examination of within-population breeding structure (VAN DEN BUSSCHE et al. 1987). Negative FIS values were obtained for TRFER, PGM-2, and HK, reflecting fewer homozygous than expected under random mating (CHESSER 1983; RYMAN et al. 1980).

Discussion

These samples show levels of genetic variation typical of fossorial rodents (NEVO 1979). Mean heterozygosity and polymorphism are within the ranges obtained in *Geomys tropicalis* (BOHLIN and ZIMMERMAN 1982), *Thomomys umbrinus* (HAFNER et al. 1987), and *Thomomys talpoides* (NEVO et al. 1974).
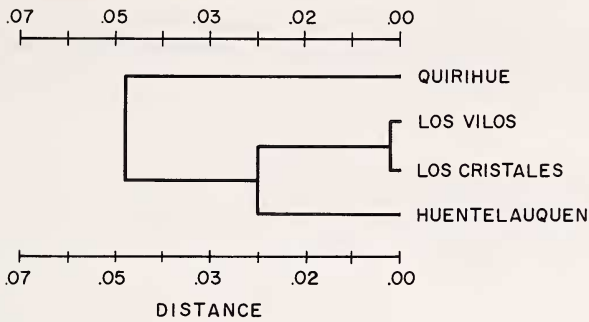
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 .07      .05      .03      .02      .00
 ├───┼───┼───┼───┼───┼───┼───┤
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QUIRIHUE

LOS VILOS

LOS CRISTALES

HUENTELAUQUEN

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 ├───┼───┼───┼───┼───┼───┼───┤
 .07      .05      .03      .02      .00
          DISTANCE
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Fig. 2. Distance phenogram between populations of *Spalacopus cyanus*. The unweighted pair-group algorithm was used to cluster Nei's genetic distances (Nei 1978). Cophenetic correlation coefficient: 0.80

Low H has been invoked to be an adaptive strategy in the relatively uniform subterranean environment (Nevo 1990; Nevo and Shaw 1972). Although a regime of "homoselection" could be claimed for the excess of monomorphic loci in the Quirihue population, interpopulation genic differentiation argues against this explanation. If environmental heterogeneity alone were sufficient to maintain genetic variability, the same alleles under the niche-width hypothesis should be promoted by directional selection in all populations, regardless of distance (Patton and Feder 1978). High levels of homozygosity and low levels of polymorphism in the Quirihue sample are better explained as stemming from founder events affecting a geographical isolate where a decrease in the frequency of heterozygotes is expected to occur. A similar effect, but due to a small sample size is observed in the Huentelauquén sample where extensive inbreeding increases homozygosity while the genus' colonial behavior further reduces the effective population size (Reise and Gallardo 1989b). This results in a pattern that mimics homoselection, especially in loci segregating at frequencies close to zero or one (Gallardo and Köhler 1992).

Gregarious *Spalacopus* occupies a common burrow, establishing large populations formed by many small, nomadic colonies where no less than three generations coexist (Reig 1970). This nomadic behavior is thought to be a consequence of their herbivory, limited to bulbs of geophytes (Reig 1970). From these observations, high vagility has been inferred, and a concomitant enhancement of gene flow is expected to occur. Accordingly, chromosome uniformity and low taxonomic diversification are explained by the animal's feeding behavior (Reig et al. 1972). Nevertheless, diet resources are not depleted by feeding activities; on the contrary, bulb regeneration seems to be facilitated through seed germination suggesting coevolution of geophytes and *S. c. cyanus* (Contreras and Gutiérrez 1991). A similar feeding dynamic was observed in *S. c. maulinus* feeding on *Dioscorea longipes*. Here, regermination induces a recurrent pattern of revisiting old dwelling areas, reversing the animals' nomadic habitus, thus arguing against high vagility (Reise and Gallardo 1989b). Apparently, genetic drift plays a significant role in shaping the genetic structure of these populations. As the effective population size is small, local extinctions and concomitant erosion of the genetic variation by historical bottlenecks are likely to occur (Maruyama and Fuerst 1985; Wade and McCauley 1988).

These *Spalacopus* populations occur in descrete patches where the dynamics of finite populations involve the internal demographic potential (Lande 1987) and external factors stemming from habitat fragmentation (Hastings and Wolin 1987; Hanski 1991). Accordingly, it seems that gene flow is efficient to maintain genetic integrity only in short distances where habitat continuity and the distribution of suitable soil types allows it. On a larger geographic scale, interpopulation genetic cohesion is affected by chance events

related to physiographic features (REISE and GALLARDO 1989a). Thus, long distance gene flow is affected by stochastic events acting in a long-term historical scale within finite populations, and thus may not be correlated at present with actual distances (PATTON and YANG 1977).

Acknowledgements

Zusammenfassung

Genetische Divergenz bei Spalacopus cyanus (Rodentia, Octodontidae)

Die elektrophoretisch ermittelte Proteinvariation von 4 Populationen von *Spalacopus cyanus* ließ sich in 23 Loci kodifizieren. Mittlerer Polymorphismus und mittlere Heterozygose betrugen 18,5 % bzw. 5,8 %. Es wurde ein geringer Grad an interpopulationärer genetischer Differenzierung gefunden (S = 0,93; D = 0,032). Der Fixierungs-Index zeigte im Gegensatz zur Annahme, daß bei *Spalacopus* extensive Durchmischung vorherrscht, ein beträchtliches Ausmaß an demischer Strukturierung (FST = 0,273). Der Grad allozymischer interpopulationärer Differenzen stützt nicht die Hypothese der Variation von Nischen-Weiten. Das Ausmaß an genetischer Variation beruht auf dem Isolationsgrad zwischen lokalen Populationen. Isolierung ist durch physiographische Gegebenheiten und historische Chancenereignisse beeinflußt und ist nicht mit der geographischen Distanz per se zu korrelieren.

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